
Studies on Blood Protozoal Diseases with Biochemical and Histopathological Changes in Some Cultured and Wild Freshwater Fishes

Heba I. Abdel-Mawla*, Osman H. A.***, Amina Dessoki*** and Amal A. Atwa*

*Animal Health Res. Institute (Ismailia Branch), ** Department of Hydrobiology, National Research Center, Giza and Dept of Pathology, Fac. of Vet. Med., Suez Canal Univ.***

Abstract

A total number of 350 cultured and wild freshwater fishes of different body weights. They were randomly collected from kafer El-sheikh cultured fish farms and also from various water sources for detection of blood protozoal diseases. The collected fishes were represented as 100 wild *Clarias gariepinus*, 100 wild *Oreochromis niloticus*, 75 cultured *O. niloticus* and 75 cultured *Cyprinus carpio*. The cultured and wild fishes were collected seasonally. The main clinical picture of the infested fishes with blood protozoal parasites were abrasions on the external body surface, off food, emaciation, dullness, respiratory manifestation. Pale gills and external body surface with distended abdomen . The total prevalence of blood parasites in all examined fishes was 29.7 %. The highest rate of infestation of *Trypanosoma mukasai* and *Babesioma clarias* was in wild *C. gariepinus*. *Babesioma tilapae* in cultured & wild tilapia and *Babesioma sp.* in cultured *C. carpio* showed the highest infestation in summer, summer and spring seasons respectively. Protozoal blood parasites showed dramatic changes in haemogram, leukogram picture, total proteins, albumin, globulin blood level, glucose and creatinine on all examined fishes. The infected fishes with *Trypanosoma* and *Babesioma sp.* showed different histopathological changes in liver, kidneys, spleen, gills, skin and musculature.

Key words: Trypanosomiasis - Babesiosomiasis - *Clarias gariepinus* - *Oreochromis niloticus* - *Cyprinus carpio* - Haematological parameters - serum biochemical analysis- histopathological changes

Introduction

Protozoan parasites had been known for many years to infect several groups of fishes and cause great damage to their host fish. Protozoa can attack and live in fish blood resulting in various dangerous outbreaks. Sleeping Sickness disease or trypanosomiasis in fish caused by haemo-flagellates of genus *Trypanosoma* which regarded as one of the most important economical internal protozoal disease affecting freshwater fishes and transmitted by leeches as vectors (*Muhammad et al., 2017*). In many instances individuals of protozoan parasites provoke the secondary infection of other pathogens like viruses, fungi and bacteria and are the most dangerous parasitic group that probably cause great losses in fish cultures (*Eissa, 2002 and El-Tntawy and El-Sherbiny, 2010*).

In some carp, it became undetectable in blood and also in internal organs while in others low numbers found in the blood for only years as chronic case (*Overath et al. 1999 and Figueroa et al. 1999*)

Infested hybrid catfish with *trypanosoma* sp. showed hematological changes by reduction of red cell count in infected samples, increasing in blood cell count. Hemoglobin and the percentage of hematocrit were dropped in samples with *Trypanosoma* sp. infection (*Kidchakan 2005 and Supamattaya*

et al. 2008). *Kharat and Kothavade (2012)* isolated trypanosome, from *Clarias batrachus* population from Mula- Mutha River system of Pune, Northern Western Ghats, India. The prevalence of trypanosomiasis was 82.70%. Severe infection resulted in decrease in total R.B.Cs, total W.B.Cs counts and lymphocytic count has shown significant decrease due to infection. While hematological parameters revealed no significance change between infected and non-infected freshwater armored ornamental fish species from Brazil which reported by *Fujimoto et al. (2013)*.

Regarding Babesiosomiasis in fish it caused by the intra-erythrocytic sporozoan *Babesiosoma niloticus* (*Eissa et al., 1993*).

This study was aimed to determine the clinical picture of the naturally infected fishes with some blood protozoans. Recording different prevalence of blood protozoans among some freshwater fishes, determination of haematological and serum biochemical analysis. Besides, histopathological changes induced in the examined fishes.

Materials and Methods

Fishes: A total number of 350 cultured and wild freshwater fishes represented as 100 wild *Clarias gariepinus*, 100 wild *Oreochromis niloticus*, 75 cultured *O. niloticus* and 75 cultured *Cyprinus carpio* of different body weights. They were randomly collected from Kafer El-

shekh cultured fish farms and also from various water sources by the aid of fishermen for detection of some protozoal blood diseases. They were collected seasonally as 25 fish in each season and taken alive in tanks filled with their natural water.

Clinical examination: Live fishes were clinically examined for detection of any gross signs and/or any external abnormalities according to *Conroy and Hermann (1981)*. Fishes were sacrificed and subjected to postmortem examination for the detection of any internal abnormalities according to *Noga (2010)*.

Sampling & examination of blood: Blood samples were taken from caudal blood vessels of the examined fishes to make blood films for detection of protozoal parasites according to *Lucky (1977)*. Blood films were air-dried, fixed in methyl alcohol for 5 minutes and stained with diluted Giemsa stain. Impression smears prepared from the cut surfaces of liver, spleen and kidneys after blotting of excess blood were air dried, fixed in methyl alcohol and stained with diluted Giemsa stain (*Noga, 2010*).

Haematological examination: Fresh blood samples were taken from all examined groups by using plastic syringe (*Stoskopf, 1993*) and kept in Epindoorf tubes containing EDTA as anti-coagulant. Erythrocytes (RBCs) and total leukocytic count (TLC) were

performed by manual method according to *Feldman et al. (2000)* using improved Neubauer hemocytometer and Natt & Herrick solution as a diluting fluid (*Natt and Herrick, 1952*). Haemoglobin (Hb) concentration was determined using Sahli's method (*Larsen and Snieszko, 1961*). Haematocrit value (PCV) was determined according to *Stoskopf (1993)*. Differential leucocytic count were made by using fresh blood films from the investigated fish (*Schaperclaus 1992* and *Stoskopf 1993*). Red cell indices, MCV (fl) MCH (pg) MCHC (%) were calculated.

Serum biochemical analysis:

Prepared serum samples were used and analyzed for some serum components including glucose, total protein, albumin, globulin, A/G ratio, urea and creatinine. Glucose was determined according to *Werner et al. (1970)*. Total proteins were determined according to *Young (2001)*, Albumin was determined according to *Dumas and Biggs (1972)*, Globulins were estimated by subtraction the total protein than albumin. A/G ratio was calculated by dividing albumin to globulin blood value (*Kaneko et al. 1997*), Urea was determined according to *Numann et al. (1957)* and Determination of creatinine according to *Henry et al. (1974)*.

Histopathological examination: Specimens from the haemopoietic organs (Skin, musculature, liver, spleen and kidneys) and gills of infected fish were collected. They

were fixed, dehydrated, mounted and stained according to *Carleton (1976)*.

Statistical analysis were performed using SPSS ver.17 software package.

Results and Discussion

Clinical picture: The most clinical signs noticed on the examined *C. gariepinus* were emaciated bodies, paleness of gills and accessory organs with increased mucous secretion. Some fish suffered from abnormal coloration with abrasions of skin, eroded fins and wounds at the base of the dorsal and caudal fins. In advanced infections, fish were laying on the bottom of aquaria, with dullness, off food and loss of escape reflex. Meanwhile, paleness of liver, kidneys with enlargement and congestion of spleen in addition of watery blood. (**Plate1, A and B**). In case of infected Cultured and wild tilapia (*O. niloticus*) and Common carp (*C. carpio*), they showed emaciation, stop feeding, sluggish movements, paleness, gulping of air, swimming in spiral movement and loss of escape reflex. Paleness of the heart, enlargement of the kidneys and congestion of the liver (**Plate1, C and D**). This finding is similar to that obtained by *Eissa et al. (1996)*, *Noga (1996)*, *Essam and El-Khateib (2004)*, *Eissa et al. (2008)*, and *Osman et al. (2009)*.

Parasitological findings: From the morphological and parasitological examinations, this parasite was

belonged to **Family:** Trypanosomatidae, **Species:** *Trypanosoma mukasai* (**Plate 2, A**). This finding nearly similar to the description of *Chong (2005)* and *Osman et al. (2009)*. Another parasites was belonged to **Class:** Sporozoa, **Species:** *Babesioma clarias* isolated from *C.gariepinus* and *Babesioma tilapae* isolated from *O. niloticus* was nearly similar to the description of *Nico et al. (2003)* (**Plate 2 B, C & D**).

Seasonal prevalence of blood protozoal diseases among examined fishes: The total prevalence in both wild *C. garepinus*, and *O. niloticus* were represented as 42 and 24% respectively. These are higher than cultured *C. carpio* and *O.niloticus* as they were 22.7 and 28% respectively. These results are nearly similar to that reported by *Kidchakan (2005)*, *El-Mowafy (2008)*, *Osman et al.(2009)* and *Borges et al.(2016)*.

Trypanosoma mukasai recorded the highest rate of infection during winter in *C. gariepinus* and the lowest rate was recorded in autumn. These results confirmed by *EL-Mowafy (2008)* who mentioned that the examined *C. gariepinus* showed higher infection in winter than in summer. He isolated trypanosoma sp. with higher prevalence than other fish species and disagree with *El-Gayar & Aly (2013)* who isolated *Trypanosome tilapiae* with a percentage 2.9% from random group of about 250 *O. niloticus*

while *C. gariepinus* was free from trypanosome. *Babesioma clari* as recorded its highest rate of infestation during summer while it recorded the lowest rate during winter. Also, *Babesioma tilapiae* among cultured and wild *O. niloticus* recorded its highest rate during summer while it recorded the lowest rate of infestation during autumn and winter respectively **Negm El-Din and Davies 1999 and Eissa et al., 2008**).

Haematological findings: The present results showed anemia in Trypanosoma and Babesiosoma infected groups in *C. gariepinus*, in comparison with control. Anemia was observed in *C. carpio* and *O. niloticus* parasitized by Babesiosoma sp. In the same line, **Kidchakan (2005)** reported decreased erythrocyte, hemoglobin and PCV in catfish infested with trypanosome sp. **El-Khatib and Elias (2003), El-Mowafy (2008) and Supamattaya et al. (2008)** observed anemia in *C. gariepinus* and *C. carpio* infested with trypanosoma sp. In this study, Leukogram showed, non-significant changes in *C. gariepinus* infected with Trypanosoma sp. and Babesiosoma sp. as well as in *C. carpio* infected with Babesiosoma sp. while leukocytosis was observed in *O. niloticus* suffer from babesiosomiasis.

The results agree with **El-Khatib and Elias (2003)** and **Adawy and Deep (2007)** who recorded leukocytosis in *C. gariepinus* parasitized by trypanosoma sp.

Eosinophilia was recorded in *C. gariepinus* and *C. carpio* infected with trypanosomiasis and babesiomiasis as the results recorded by **Anisworth, (1992)**

Biochemical parameters: The present study revealed that hyperproteinemia and hyperalbuminemia as well as hyperglobulinemia in all investigated groups in compare with control similarly to that obtained by **Gehad (2003)** and **Supamattaya et al. (2008)** who mentioned that the parasitic infestation caused hematological changes as reduction in red blood cells (anemia) and white blood cells increased (leukocytosis) in infected samples respectively. Hemoglobin and hematocrit were dropped (anemia) in samples with infestation. Hyperglobulinemia in the present study could be attributed to enhanced immune conditions in the infected fishes. This conclusion confirmed by lymphophilia and Eosinophilia in peripheral blood and histopathologically by congestion, perivascular edema and depletion of lymphoid follicles in *C. gariepinus* parasitized by trypanosoma sp. and babesiosoma sp. (**Gehad, 2003 and Osman et al., 2009**).

Glucose blood level in the present study showed hyperglycemia in *C. gariepinus*, *O. niloticus* and *C. carpio* infected with trypanosoma and babesiosoma sp. as the results obtained by **Joshi (1982)** who reported hyperglycemia in catfish

parasitized by trypanosoma species. The increase in blood glucose levels may be due to stress induced by parasitic infection. Urea and creatinine blood level are elevated only in *C. gariepinus* infected with Trypanosoma sp. and insignificant in babesioma infected groups. Urea in fish is produced by liver, it is excreted primarily by the gills rather more the kidney (Stoskopf, 1993). The elevation of urea in the present study may be attributed to gill and liver dysfunction.

Histopathological findings: Liver showed congestion of blood vessels, degeneration and necrotic changes. Parenchymal edema and hemosiderosis was also observed. Kidneys showed necrobiotic change, vaculation, leukocytic infiltration and mild hemosedrosis. Spleen showed congestion,

perivascular edema, depletion of lymphocytes and hemosiderosis. Gills showed hyperplasia of epithelial lining of secondary lamellae, hyperplasia of blood vessels and edema. Skin of *C. gariepinus* showed focal distribution of melanin pigment with melanocytes with infiltration of inflammatory cells while skin of infected *O. niloticus* showed melanin pigment with melanocytes distributed in diffused accumulation with infiltration of inflammatory cells. Musculature of *C. carpio* showing hyalination of muscle bundles with infiltration of inflammatory cells with edema (Plate 3 & 4). This finding nearly in agreement with that recorded by Supamattaya et al. (2008) and El-Gayar and Aly (2013).

Table (1): Total and Seasonal prevalence of blood protozoal diseases in examined fishes.

Fishes	Total No. of exam	Total No. of inf.	%	Season	No. of examined fish	Blood protozoan			
						Trypanosoma		Babesioma	
						No.	%	No.	%
Wild <i>C. gariepinus</i>	100	42	42	spring	25	-----	-----	3	12
				summer	25	10	40	6	24
				autumn	25	7	28	3	12
				winter	25	12	48	1	4
Wild <i>O. niloticus</i>	100	24	24	spring	25	-----	-----	8	32
				summer	25	-----	-----	9	36
				autumn	25	-----	-----	5	20
				winter	25	-----	-----	2	8
Cultured <i>O. niloticus</i>	75	21	28	spring	25	-----	-----	7	28
				summer	25	-----	-----	10	40
				autumn	25	-----	-----	4	16
Cultured <i>C. carpio</i>	75	17	22.7	spring	25	-----	-----	7	28
				summer	25	-----	-----	6	24
				autumn	25	-----	-----	4	16
Total	350	104	29.7	-----	350	29	8.3	75	21.4

Table (2): Showing some hematological parameters (Mean±S.E) in examined fishes infected with blood protozoan.

Fishes	group	RBCs 10 ⁶ /μL	Hb g/dl	PCV %	MCV FL	MCH cp	MCHC %
<i>C. gariepinus</i>	Control	3.42±0.14	8.91±0.41	29.10±1.15	120.2±3.82	36.9±1.85	31.6±1.05
	T	1.44±0.12*	6.59±0.55*	24.23±1.44*	111.3±3.03*	33.03±1.45	27.2±1.03*
<i>O. niloticus</i>	Control	1.85±0.13	7.94±0.12	26.5±3.2	267.4±6.45	45.6±1.43	17.23±0.13
	B	0.87±0.08*	3.87±0.21*	22.45±1.22*	225.7±9.25	42.67±2.67	14.55±0.32
<i>C. carpio</i>	Control	1.91±0.12	7.34±0.32	27.8±1.11	156±4.56	45.4±1.67	26.5±1.35
	B	0.78±0.15*	4.87±0.43*	21.85±1.14*	144±6.56	33.5±1.78	19.1±0.68

T: Trypanosoma B: Babesiosis Mean ± SE *Significant at P> 0.05

Table (3): Total and differential leucocytic count (Mean±S.E) in the examined fishes infected with blood protozoan.

Fishes	Group	TLC 10 ³ /μL	Granulocytes			Agranulocytes	
			Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes
<i>C. gariepinus</i>	Control	19.93±2.14	4.45±0.87	1.34±0.05	0.08±0.04	13.54±1.07	0.52±0.35
	T	27.88±3.23*	5.57±0.76*	4.43±0.04*	0.95±0.12	15.57±1.33*	1.36±0.44*
<i>O. niloticus</i>	Control	33.10±2.56	9.05±0.86	10.78±0.08	0.0±0.22	12.16±0.34	1.56±0.32
	B	38.00±3.27*	9.68±4.22	12.64±0.09*	1.0±0.23*	13.65±0.43	1.97±0.32
<i>C. carpio</i>	Control	31.19±2.43	6.26±0.87	3.67±0.06	9.65±0.65	11.16±1.23	1.45±0.24
	B	36.23±2.89*	6.39±0.45	5.00±0.06*	11.00±0.45*	12.34±1.14	1.48±0.24

T: Trypanosoma B: Babesiosis Mean ± SE *Significant at P> 0.05

Table (4): Serum chemical parameters in examined fishes infected with blood protozoan.

Fishes	group	Total proteins	Albumin g/dl	Globulin s g/dl	A/G ratio	Glucose g/dl	Urea g/dl	Creatinine mg/dl
<i>C. gariepinus</i>	control	6.44 ±0.45	3.45 ±0.12	2.99 ±0.19	1.15 ±0.12	88.1±4.5	13.1±1.2	1.45 ±0.23
	T	7.45 ±0.23*	3.01 ±0.09	4.44 ±0.13*	0.68 ±0.14	119.8 ±5.3*	19.3 ±1.3*	1.87 ±0.21
<i>O. niloticus</i>	control	3.23 ±0.34	1.65 ±0.13	1.58 ±0.12	1.04 ±0.90	118 ±4.6	8.9 ±0.67	0.95 ±0.03
	B	4.98 ±0.44	1.11 ±0.07	3.87 ±0.23*	0.28 ±0.05	155.4 ±6.7*	9.2 ±0.5	1.66 ±0.07*
<i>C. carpio</i>	control	3.98 ±0.55	1.95 ±0.13	2.03 ±0.14	0.47 ±0.11	77.5 ±3.5	11.3 ±0.68	0.87 ±0.09
	B	4.65 ±0.36*	1.87 ±0.10*	3.78 ±0.12*	0.49 ±0.03	116.3 ±7.2*	12.9 ±0.56	1.88 ±0.13*

T: Trypanosoma B: Babesiosis Mean ± SE *Significant at P> 0.05

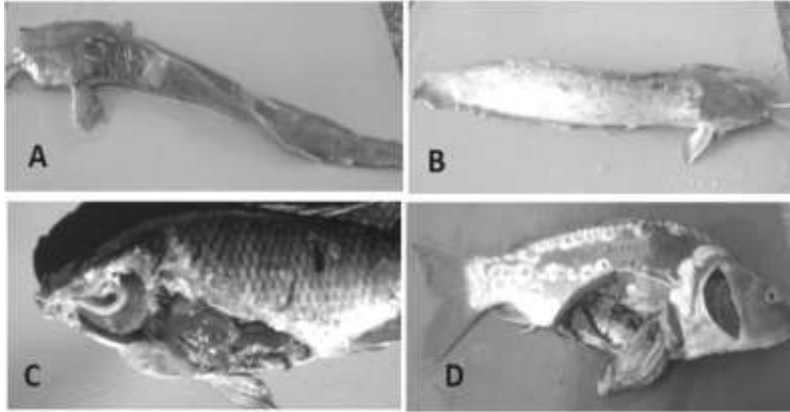


Plate (1): **A:** infected *C. gariepinus* showing severe emaciation with eroded fins **B:** skin abrasions, eroded caudal fins, excessive mucous secretion and emaciation. **C:** *O. niloticus* suffered from congestion and inflammation of gills and some internal organs. **D:** *C. carpio* with congested gills, internal organs and bloody serous fluids.

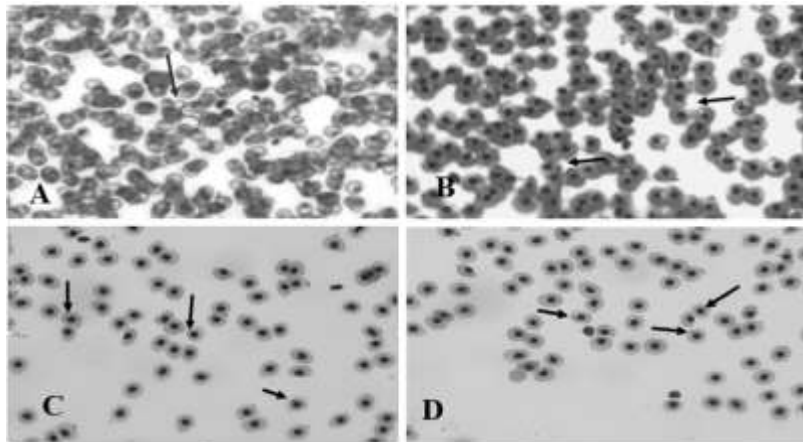


Plate (2): **A:** blood film of *C. gariepinus* displaying *T. mukasai* (X400). **B:** blood film of *C. gariepinus* displaying *Babesioma clarias* (arrows) (X1000). **C:** blood film of *O. niloticus* displaying *Babesioma tilapiae* (arrows) (X1000). **D:** blood film of *C. carpio* displaying crescentic shape of *Babesioma* sp. (arrows) (X1000).

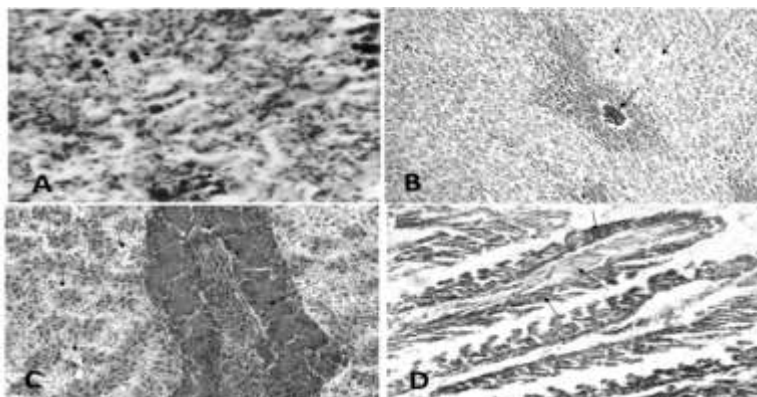


Plate (3): *C. gariepinus* **A:** Spleen showing congestion of red pulp and focal aggregation of melano- macrophage with hemosiderosis. X 400 **B:** Hepatopancrease showing congestion of the hepatic sinusoids and vacuolar degeneration of the hepatic cells lysis of hepatocytes were noticed other hepatic cells were necrotic X 200. **C:** Mild vacuolation and cloudy swelling with mild degeneration in hepatocytes X 200. **D:** hyperplasia of secondary gill lamellae with infiltration of inflammatory cells with congestion of gill blood vessels X 200.

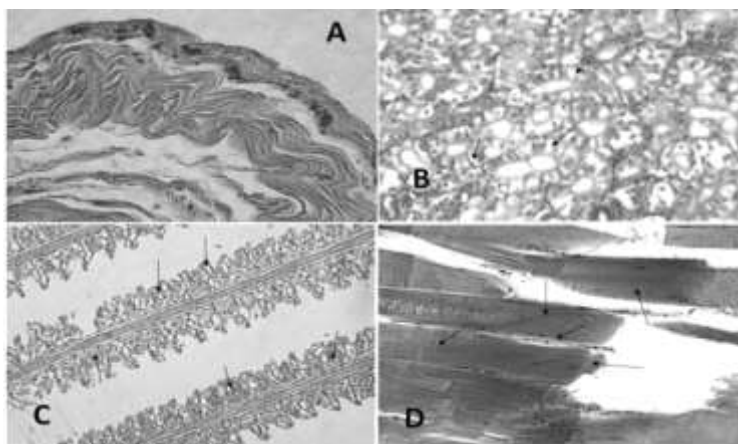


Plate (4): *C. gariepinus* **A:** focal distribution of melanin pigment with melanocytes with infiltration of inflammatory cells X 400. *O.niloticus* **B:** kidney showing vacuolar degeneration of the renal tubular epithelium with lysis of the cells together with peritubular aggregation of melanophores X 400. **C:** Gills showing hyperplasia of secondary gill lamellae with infiltration of inflammatory cells X 200. Common carp **D:** hyperplasia of secondary gill lamellae, with infiltration of inflammatory cells and congestion gill vascular vessels X 200.

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دراسة على أمراض أوليات الدم مع التغيرات الكيميائية الحيوية والهستوباثولوجية في بعض أسماك المياه العذبة المستزرعة والحررة

هبة إبراهيم عبد المولى* - حسين عثمان** - أمينة دسوقي*** - امل احمد محمد عطوه*
 *قسم امراض الاسماك معهد بحوث الصحة الحيوانية- فرع الاسماعيلية
 **قسم الهيدروبيولوجي-المركز القومي للبحوث- الجيزة
 ***قسم الباثولوجيا- كلية الطب البيطري-جامعة قناة السويس

أجريت هذه الدراسة على عدد 350 سمكة من اسماك المياه العذبة المستزرعة والحررة ذات الأوزان المختلفة وقد تم جمعها من تفرعات النيل ومن المزارع الخاصة بمحافظة كفر الشيخ للكشف عن الأمراض الطفيلية الاولية في الدم. مثلت الاسماك المجمعه الحررة كانت بواقع 100 سمكه من القرموط الافريقي و 100 سمكة من البلطي النيلي والاسماك المستزرعه كانت بواقع 75 سمكة بلطي نيلي و75 سمكة من المبروك العادي وقد تم تجميعها موسميا.

كانت الصورة الاكلينيكية للاسماك المصابة بأوليات الدم الطفيلية (التريبانوسوما والباييزوسوما) هي هزال عام وفقدان الشهية وعلامات تنفسية وسحجات على الجلد مع شحوب في لون الجلد والخياشيم وكذلك فقدان الاسماك المصابة لظاهرة الهروب. كانت نسبة الاصابة الكلية بأوليات الدم الطفيليه في جميع الاسماك التي تم فحصها 29.7%. كان اعلى معدل إصابة بالتريبانوسوما موكاساي، الباييزوسوما كلاريس في أسماك القراميط الحررة. الباييزوسوما تيلابي في البلطي المستزرع والحر و الباييزوسوما سبيشتر في المبروك العادي سجلوا اعلى نسبة اصابة في فصول الصيف والصيف والربيع على التوالي.

اظهرت الاصابة بأوليات الدم تغيرات جذرية في صورة مكونات الدم ، البروتينات الكلية، الألبومين، ومستوى الجلوبيولين في الدم، الجلوكوز والكرياتينين في جميع الأسماك المصابه. الاسماك المصابه بالتريبانوسوما و الباييزوسوما أظهرت تغيرات هستوباثولوجية مختلفة في الكبد والكلى والطحال والخياشيم والجلد والعضلات.